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INVITRO ANTIOXIDANT ACTIVITY OF *ALBIZIA LEBBECK* (L) BENTH

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ABSTRACT

Medicinal plants are traditionally used in folk medicine as natural healing remedies with therapeutic effects such as prevention of cardiovascular diseases or reducing the risk of cancer. Medicinal plants are valuable because of the presence of chemical substances and antioxidants properties. The objective of the present work is to study the *invitro* antioxidant activity by DPPH scavenging, Nitric oxide assay, hydrogen peroxide, sulphur oxide and total antioxidant assays. The results revealed that the hydroalcoholic leaves extracts of *Albizia lebbeck* showed a strong antioxidant activity, reducing power ability and free radical scavenging when compared to the standard such as Ascorbic acid.

KEYWORDS: *Albizia lebbeck,* Antioxidant Activity, Free Radical Scavenging and Reducing Power Ability

INTRODUCTION

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability and nutritional supplements and possess strong and potent antioxidant activities (Sumino *et al.,* 2002).

Medicinal plant parts (roots, leaves, branches/ stems, barks, flowers and fruits) are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. (Cai *et al.,* 2004; Kahkonen *et al.,* 1999; Larson, 1988). They have multiple biological effects including antioxidant activity (Tapiero *et al*., 2002).

Medicinal plants are reported to be rich in antioxidants, namely, polyphenols, flavonoids, vitamin A,C,E and several other constituents, which are necessary for maintaining good health and useful for therapeutic purposes against various diseases(Scalbert *et al*., 2005).Medicinal plants are gaining a lot of importance as an alternate medicine against therapy and prevention from various diseases. Besides phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc (Miller, 1996). As crude extracts of herbs and spices and other plant materials, rich in phenolics are increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative can enzymes and anti-inflammatory action (Vinay *et al*., 2010).

The medicinal plants research is presently intensively focused on the identification of naturally occurring anticarcinogens, which were found in certain plants. The interest in these natural medicines is mainly due to the fact that diseases such as cancer are still difficult to cure. Therefore, there is a great scientific effort to delay the process of carcinogenesis and to reduce the morbidity and mortality of cancer. In addition, the usage of potent biologically active components of medicinal plants as chemo preventive agents seems to be very promising.

Medicinal plants and their components possess a range of beneficial preventive properties. They show many promising effects for various health problems, such cold, cough, throat irritations, stomachache, indigestion, and gastrointestinal diseases and have also positive protecting activities such as sedative, antiviral, antiinflammatory, antiseptic, heapatopretective, antihyperglycemic, and immunostimulating (Sona Skrovankova *et al*., 2010).

Antioxidants refers to a compound that can delay or inhibits oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen. They act by one or more of the following mechanisms: reducing activity, free- scavenging, potential complexion of pro-oxidant metals and quenching of singlet oxygen. Epidemiological protect the human body against damage by ROS. The consumption of natural antioxidant was reported to have potential health benefits. (Di Carlo *et al*., 1999, Pulido *et al*., 2000, Sumino *et al*., 2002, Suganya Tachakittirungrod *et al*., 2007). Though humans have excellent defense mechanism to overcome oxidative stress related diseases caused by ROS, which is however affected by several factors, *e.g.*, age, diet, health status of individual (Chun, Kim & Lee, 2003). A proper equilibrium between the ROS generation in humans and components of defense system is needed by supplementing antioxidants via diet or medicine (Yu *et al*., 2002) to prevent diseases related to chronic oxidative damages in tissues and cells and are thus believed to protect against cancer, cardiovascular diseases and could delay the aging process (Ross & Kasum, 2002). Synthetic antioxidant supplementation, namely butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT), tertiary butyl hydroquinone, are reported to exhibit tumor forming activities and hence carry health risks (Kahl & kappus,1993). While antioxidants from plant sources such as vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs are bioavailable, safe for human consumption and consequently explored (Liu & Ng, 2000; Pietta, 2000; Rababah *et al*., 2004)

The Antioxidant activity of plant products can be mainly ascribed to the presence of phenolic compounds (Heim *et al*., 2002). Plant synthesis phenolic and flavonoid compounds for its own defense system against ROS. The Phenolic compounds acts as reducing agent due to their hydrogen donating and single oxygen quenching ability, which not only prevent generation of oxidant free radicals and reactive species but also scavenge free radicals (Seyoum *et al*., 2006). Phenolic compounds are not evenly distributed in plant parts. The Antioxidant contents are measured as total poly phenolic content and total flavonoid content; and the antioxidant content are measured as DPPH (1,1-DiPhenyl-1-Picryl hydrazyl) free radical (DPPH) scavenging ability and oxygen radical scavenging activities, ferric reducing ability, metal chelating and in hibilation of lipid peroxidation capacity (Sultana *et al*., 2007).

The human body possess innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Vitamin C, E, selenium, carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Apart from these, plant secondary metabolities such as flavonoids and terpenoids play an important role in the defence against free radicals (Devasagayam & Sainis, 2002; Govindrajan *et al*, 2005; Park & Pezzutto, 2002).

Albizia lebbeck, known locally as siris tree and in Tamil it is called as vaagei, is an unarmed deciduous woody tree, 12- 21 m in height, having pale bark with glabrous young shoots. It is cultivated in many parts of India in farmlands, along roadsides, on irrigated plantation, along rivers and as an ornamental plant in garden due to its pleasant appearance. It is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds, grow wild. Plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa (Kirtikar and Basu, 1980). The present investigation on the plant is on antioxidant activity.

MATERIAL AND METHODS

Collection of Plant Material

The leaves and barks of *Albizzia lebbeck* were collected and the specimen were deposited in the Alpha Omega Hi- Tech Bio research centre. The fresh leaves and barks of *Albizzia lebbeck* were authenticated by Dr. A. Balasubramanian, ABS Botanical conservation, Research & Training Centre, Salem (Dt).

Extraction of the Plant Material

The fresh plant materials were washed with running tap water and shade dried. The leaves and barks were crushed to coarse powder by grinder. These coarse powders (25g) were then subjected to successive extraction in 250ml of each solvent (hexane, ethyl acetate and hydroalcohol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100. IC50 value was calculated using Graph pad prism 5.0.

Nitric Oxide Radical Scavenging Activity

Nitric oxide radical scavenging activity was measured spectrophotometrically (Govindharajan *et al*., 2003). 1.0 ml of Sodium nitroprusside (5 mmol) in phosphate buffer (p H 7.4, 0.1 M) was mixed with different concentrations of the extract (100 – 500 microgram/ml in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25^{o} c for two hours. At the end of second hour 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% of naphthyl ethylene diamins dihydrochloride) The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphtylethylene diamine dihydrochloride was measured at 546 nm. Control tube contain all chemicals except plant extract.

Hydrogen Peroxide Radical Scavenging Activity

The hydrogen peroxide radical scavenging activity of the test sample was estimated by following the method of Ruch *et al*., (1989). A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 200 µl of sample containing different concentrations were mixed with 0.6 ml of H_2O_2 solution. Absorbance of H_2O_2 was determined

10 minutes later against a blank solution containing phosphate buffer without H_2O_2. A test tube containing 200 µl of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the test sample was studied using the method of Liu *et al*., (1997) with slight modifications. Superoxide radicals are generated in phenazine methosulphate (PMS) - (Nicotinamide adenine dinucletide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200.0 µl of test samples of different concentrations were taken in a series of test tube. Superoxide radicals were generated by 1.0 ml of Tris-HCl buffer (16.0 mM, pH-8.0), 1.0 ml of NBT (50.0 µM), 1.0 ml NADH (78.0 µM) solution and 1.0 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris HCl buffer was also processed in the same way without test sample. Different concentration of ascorbic acid was used as reference compound.

RESULTS AND DISCUSSIONS

The DPPH test is a very convenient method for screening small antioxidant molecules because the reaction can be observed visually using common TLC and dot - blot techniques, and also its intensity can be analyzed by simple spectrophotometric (Sanchez - Moreno *et al*., 1998; Soler-Rivas *et al*., 2000). The DPPH radical is scavenged by antioxidants through the donation molecule. The antioxidant radicals formed are stabilized through the formation of non-radical products. The percentage of DPPH radical scavenging activity of leaf extract (14.87%) is somewhat better than the ascorbic acid (14.84%) (Standard) when compared with bark extracts (12.95%).

Nitric Oxide: The percentage inhibition in Nitric oxide in leaf hydro alcoholic extracts is about 13.62% and bark is 11.84% where as in Ascorbic acid (Standard) is found to be 11.05%. The scavenging ability of hydro alcohol extracts of leaf and bark on H_2O_2 and Superoxide. The results revealed that the leaf extracts and bark extracts exhibited 28.09% and 21.63% respectively on the other hand Ascorbic acid exhibited 21.47% H_2O_2 scavenging activity. From the results it was observed that the scavenging activity values on H_2O_2 increases than Ascorbic acid. The results observed for Superoxide free radicals were found that leaf hydro alcohol extract (36.9%) showed much better inhibition than bark (22.8%) & Standard Ascorbic acid (25.4%).

Antioxidant Activity of *Albizzia lebbeck* (Benth)

Table 1

Samples	Percentage of Inhibition (%)			
	DPPH	Super Oxide	H_2O_2	Nitric Oxide
Leaf Hydro alcohol	14.87	36.9	28.09	13.62
Bark hydro alcohol	12.95	22.8	21.63	11.84
Standard				
Ascorbic acid	14.84	25.4	21.47	11.05

Several studies are focused on the relationship between the antioxidant activity of the phenolics compounds, as hydrogen donating free radical scavengers and their chemical structure. It has been shown that the presence of the ACH@CHACOOH group in the hydroxylated cinnamates ensures greater H-donating ability and subsequent radical

stabilization than the carboxylate group in the hydroxy benzoates (Rice-Evans, Miller, & Paganga, 1996). Phytochemicals especially plant phenolics constitute a major group of compounds that act as primary antioxidants (Hatano *et al*., 1989). They have high redox potentials which allow them act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et al*., 1999). The delocalization of electrons over the phenolics and stabilization by the resonance effect of the aromatic nucleus prevents the continuation of the free radical chain reaction (Tsao and Akhtar, 2005). The antioxidant effects of the extract may be due to its phenolic content. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the in vitro general antioxidant activity of pure compounds as well as plant extracts (Koleva *et al*., 2002; Goncalves *et al*., 2005). Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases and inflammatory conditions Cancer and ageing (Marx, 1987).

Antioxidants mar offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent diseases (Braugghler, 1986). Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. (Ialenti, 1993). In the present study, the nitrite produced by the incubation of solution of Sodium nitroprusside in standard phosphate buffer at 25^{o}C was reduced by the leaf hydroalcoholic extracts. This may be due to the antioxidant principles in the extract which compete with O_2 to react with nitricoxide thereby inhibiting the generation of nitrite.

H_2O_2 itself is not very be reactive, but it can sometimes toxic to cell because of it may give rise to hydroxyl radical in the cell (Halliwell, 1991). Thus, the removing of H2O2 is very important for antioxidant defense in cell (or) food system. Superoxide radical is considered a major biological source of reactive oxygen species (Alves *et al*., 2010). Although Superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet O_2, both of which contribute to oxidative stress (Meywr *et al*., 1995). Superoxide dismutase catalyses the dismutation of the highly reactive superoxide anion to O_2 and H_2O_2 (Kamalakkannan, 2003). Superoxide anion is the first reduction product of O_2 (Ray 2002). This is the measured in terms of the inhibition of generation of O_2.

CONCLUSIONS

Since the hydroalcoholic extract of leaf showed good antioxidant activity. The plant extracts and their active components could emerge as natural antioxidants, alternative drugs or serve as starting points for synthesizing more effective antioxidant inhibitors.

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